DOI: https://doi.org/10.31351/vol32iss1pp237-244

Assessment of Certain Hematological Indices and Interlukin-6 in Workers and Individuals Who Live Near to Crude Oil Wells in Middle Petroleum Refinery, Iraq

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Abstract

Environmental exposures to a variety of pollutant elements especially fuel waste products may result in harmful impacts on the human body physiology. The inflammatory response and the hematological system are the major affected systems. The aim of the study is to reveal the impact of oil exposure on several hematological parameters and Interleukin-6 of oil workers and to detect which parameters are more affected as prognosticators for clinical disorders. This study included three groups, fifty (50) individuals in Group I (control healthy group); fifty (50) individuals in Group II workers at oil wells; and fifty (50) individuals in Group III who live close to oil wells. Physical parameters (pulse rate, blood pressure, random blood glucose), serum interleukin 6, hematological parameters are measured. Significant rise in pulse rate, serum interleukin 6 and random blood glucose were documented in workers. Hematological parameters were adversely-affected by air pollution. There was a significant rise in platelets count in Group II. The correlation between IL-6 and certain hematological indices revealed that each of white blood cells and platelets were significantly correlated with IL-6. In conclusion, people who work or live close to oil wells area showed alterations in hematological and inflammatory parameters (increase in interleukin 6), which may be related to continuous exposure to petrol fumes.
Keywords: Oil wells, Hematological indices, Interleukin 6.

تقييم بعض مؤشرات الدم و interlukin-6 في العمال والأفراد الذين يعيشون بالقرب من آبار النفط في مصافي النفط الوسطى ، العراق

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الخلاصة

التعرض البيئي لمجموعة من عناصر التلوث البيئي وخاصة منتجات نفايات الوقود له تأثيرات ضارة على العديد من الأنظمة الفسيولوجية. الاستجابة الالتهابية والجهاز الدموي هما النظامان الاكثر تأثرا. الهدف من الدراسة هو الكشف عن تأثير التعرض لمشتقات النفط على العديد من معايير الدم و انترليوكين٦ لعمال النفط واكتشاف العوامل الأكثر تأثرًا كمنبهات للاضطرابات السريرية. اشتملت هذه الدراسة على ثلاث مجموعات ، كل منها ٥٠ شخصًا. مجموعة السيطرة ١ والمجموعة ٢ عمال في آبار النفط والمجموعة ٣ الذين يعيشون بالقرب من آبار النفط. يتم قياس التأثيرات الفيزيائية (النبض، ضغط الدم، سكر الدم) ، انترليوكين ٦ ، تحاليل الدم. لوحظ إرتفاع كبير في معدل النبض ، إنترليوكين٦ و سكر الدم في المصل. وتأثرت التحاليل الدموية سلباً بتلوث الهواء حيث وجد ارتفاعًا كبيرًا في عدد الصفائح الدموية في مرضى المجموعة ٢ و لوحظ العلاقة بين انترليوكين٦ وبعض تحاليل الدم حيث أن كل من كريات الدم البيضاء والصفائح الدموية كانت مرتبطة بشكل كبير بـ انترليوكين٦. الاستنتاج: أظهر الأشخاص الذين يعملون أو يعيشون بالقرب من منطقة آبار النفط أن التغيرات في العوامل الدموية والالتهابية قد تكون مرتبطة بالتعرض المستمر لأبخرة البترول ، مما يؤدي أيضًا إلى زيادة كبيرة في الإنترلوكين٦.
الكلمات الرئيسية: آبار النفط ، مؤشرات الدم ، الإنترلوكين

Introduction

Crude oil is a major component that workers may exposed to it which is a blend of saturated and unsaturated hydrocarbon in a liquid form, and the composition of crude oil including (Benzene, toluene, lead, oxygenates, ethyl benzene, and three isomers of xylene) [(1)]. Occupational exposure to crude oil and its product regarded as one of the most serious issues around the world today. It has a negative impact on the health and environment. The air at this workplace is made up of a variety of gases as well as tiny (solid and liquid) particles. Some compounds are derived from natural sources, while others are chemically synthesized [(2)]. The combustion of these large amounts of gases in internal combustion engines produces hazardous emissions [(3)].

Hazardous chemicals can accidentally-leak into the environment, but a variety of air pollutants considered as end products of industrial facilities and other sources, which can have adverse outcomes on human health and the ecosystem [(4)].

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Received: 17/4 /2022
Accepted: 3/7 /2022

Oil metabolites is predominantly found in the environment as a result of air pollution from oil refineries [5]. Chronic exposure to the stream of the gases and its metabolite, puts workers at risk for a variety of diseases that affect multiple systems in the body, including the renal system, immune system, cardiovascular (CV) system, and respiratory system [6].

The relative concentration of gasoline components, on the other hand is determined by the crude oil source, refining method, and production lines employed [6]. Certain volatile chemicals (xylene, toluene, ethyl benzene, and benzene) are harmful pollutants according to toxicological studies which are steam fractions of gasoline that are progressively export to the air and water [7]. When inhaled or swallowed, gasoline is quickly absorbed, uptake and dispersed in the body after absorption. Following metabolism in the liver, small percent is exhaled intact or excreted as inactive metabolites in the urine. The active metabolites are subjected to additional assays processes of toxic kinetics, such as the formation of oxidative tissue damage and reactive oxygen species (ROSs), which results in altered structure and function, as well as multi-system toxicity [8]. Benzene is converted to phenol in the liver by the enzyme cytochrome P4502E1 (CYP2E1), which is then hydroxylated to hydroquinone, catechol, and 1,2,4-benzenetriol. Because benzene metabolism produces a large number of reactive metabolites, benzene toxicity is expected to be mediated through numerous mechanisms [8]. Hematological system is one of the systems that is affected by oil exposure and its metabolites [5]. Excessive benzene exposure has been shown to affect the bone marrow, resulting in a reduction in the amount of circulating blood cells, anemia, and other health problems, aplastic anemia, thrombocytopenia, and leucopenia [9]. Also, the effect is represented by increased sensitivity to injuries, bone marrow (BM) suppression and infections caused by a lack of leucopoiesis [5]. Infections and inflammatory processes can trigger the production of cytokines, which mediate immune response [10]. Interleukin 6 (IL-6) is a significant cytokine with a variety of functions which is a low-molecular-weight protein that is mostly-released by the immune system cells (monocytes and macrophages) to control a variety of biological processes like proliferation, differentiation, and protein synthesis [11].

Researchers reported that oil may be a cause of acute lymphoblastic leukemia (AML), congenital abnormalities, and perhaps lymphocytes hematological malignancies, non-Hodgkin lymphoma (NHL) [12] [13]. The aim of the study is to reveal the impact of crude oil exposure on several hematological parameters and IL-6 of oil workers and to detect which parameters are more affected by the exposure to be considered as prognosticators and to find the correlation between these parameters.

Subjects and Methods

Study design

The study was an observational study conducted at the Middle Refineries Company (MRC) in Al-Muthanna City, South of Iraq, from the period between October 2021 to January 2022. The study was done according to strengthening the Reporting of observational studies in epidemiology (STROB) guideline and approved by the Ethical Committee of University of Basra-College of Pharmacy (293/5/3, 2021/10/21)

Participants

One hundred fifty subjects were enrolled in the study. Individuals were divided into three groups: control/non-exposed healthy persons/Group I consists of 50 persons; Group II consists of 50 workers at oil well; Group III consists of 50 persons who live close to the oil wells. The included criteria: a worker who is working for more than two years in the Middle Refinery Company (MRC) unit, workers exposed for at least (10-12) hours/day for three days/week duration period, age range between (25 - 60) years. Any individual with CV diseases, endocrine diseases, respiratory disorders and obesity were excluded from the study. Pregnancy and breast feeding are extra excluded criteria. Detailed information such as age, gender, work location and place of residence, weight, height and body mass index (BMI); individual life style (smoking, alcohol, coffee habit), disease history, surgical history, drug history were obtained by prepared questionnaire for each participant.

Measurements of the parameters

Pulse rate

Measure pulse rate of each individual by using oximeter.

Blood pressure

Measure blood pressure of each individual by classical method by aneroid sphygmomanometer.

Random blood glucose

Measure random blood glucose by Accu-check active glucometer.

Hematology Profile

Five milliliters of blood samples were obtained, place 2ml of the blood in a tube containing EDTA (ethylene diamine tetra acetic acid) to prevent blood clotting. The tube was placed in a roll-shaking mixer for few minutes to prevent clotting and then placed below needle of auto-analyzer hematology to measure hematological parameters complete blood count (CBC) i.e. white blood cell count (WBC 10^9/L), lymphocyte count [(LMY#(10^9/L)], monocyte count (Mid#10^9/L), granulocyte count (Gran# (10^9/L), lymphocyte percentage (LYM%), monocyte percentage Mid% and granulocyte percentage (Gran%) and red blood cell (RBC), hemoglobin (HGB g/dl), hematocrit (HCT%), mean corpuscular volume (MCV fl), mean corpuscular hemoglobin concentration (MCHC g/l), mean corpuscular hemoglobin (MCH pg), platelets (PLTs (10^9/L), platelet distribution width (PDW-CV%), platelet distribution width-standard deviation(PDW-SD fl) by (Ruby Hematology Analyzer, Germany) [(6)].

Immunofluorescent assay

Place three milliliters of blood in centrifuge to obtain clear serum for interleukin 6 measurement by Getein Biotech, Inc, UK. This was done by using an immunofluorescence assay (IL6 Fast Test Kit). The test was performed according to the manufacturer's instruction (14). Serum IL-6 levels were used as a predictive biomarker to confirm the role of inflammatory mediators and their link to negative pollution consequences.

Statistical analysis

One-way ANOVA with chi-square fishers exact test was utilized among groups. Tukey’s post-hoc analysis test for further assessment was used. Data were expressed as Mean± SEM with P<0.05 significance. GraphPad prism software (Version 6.0).

Results

Demographic data, as well as general history, smoking, social state and personal habits, were collected using a prepared questionnaire, which is described in Table (1). The percentage of males is higher than that of females in all groups, which showed no significant changes among the groups. To link the data and draw a clear conclusion of cause and effect, smoking and coffee consumption patterns were documented. There were significant differences (P>0.05) among the groups in such habits, with greatest percentage of smokers (40%) in Group II. Regarding coffee consumption, about 38% and 32% of the participants in both Group I and Group II workers were consumed coffee, respectively as shown in Table (1). There were no statistically-significant differences in the drug, surgical, or family histories of the participants. As well as no significant differences were found in body mass index (BMI) among the individuals of all groups as illustrated in Figure 1. The majority of the participants are of a healthy weight.

Table1. Demographics information of the participants (n=150)

Groups	State		Sex		Smoke habits		Coffee habits		Drug history		Surgical history		Family disease history	
	Married	Single	Male	Female	yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Group (I) Control	43 (86%)	7 (14%)	40 (80%)	10 (20%)	5(10%)	45(90%)	19(38%)	31(62%)	2	48	5	45	2	48
Group (II)	42 (84%)	8 (16%)	46 (92%)	4 (8%)	20(40%)	30(60%)	16(32%)	34(68%)	5	45	7	43	8	42
Group (III)	40 (80%)	10 (20%)	40 (80%)	10 (20%)	15(30%)	35(70%)	9(18%)	41(82%)	3	47	5	45	2	48
P-Value	***0.6597***		***0.0834***		***0.0014****		***0.0034****		***0.6112***		***0.6857***		***0.1284***	

***Values with percent are analyzed using One-way ANOVA with chi-square fisher exact test. Represents significant differences among groups**.

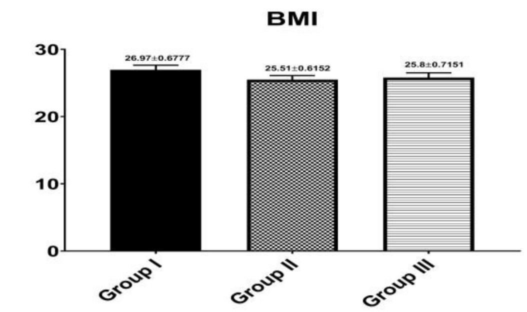

Figure 1. Body mass index (BMI) comparison among Groups. Values are expressed as Mean±SEM. No significant differences among groups.

Furthermore, pulse rate, systolic and diastolic blood pressure were measured in this study to show the potential CV effects of oil exposure or its waste products on people who work or live near the site. This test was also performed to rule out any major disease or disorder affecting the aforementioned system. There was a considerable rise in pulse rate in Groups II (85.68 ±1.58) and Group III (86.65±1.88) when compared to Group I (78.43±1.53), but it was still within normal limits. In contrast, no significant differences in systolic and diastolic blood pressure were found among groups, and the majority of them were within normal ranges, as shown in Figure 2

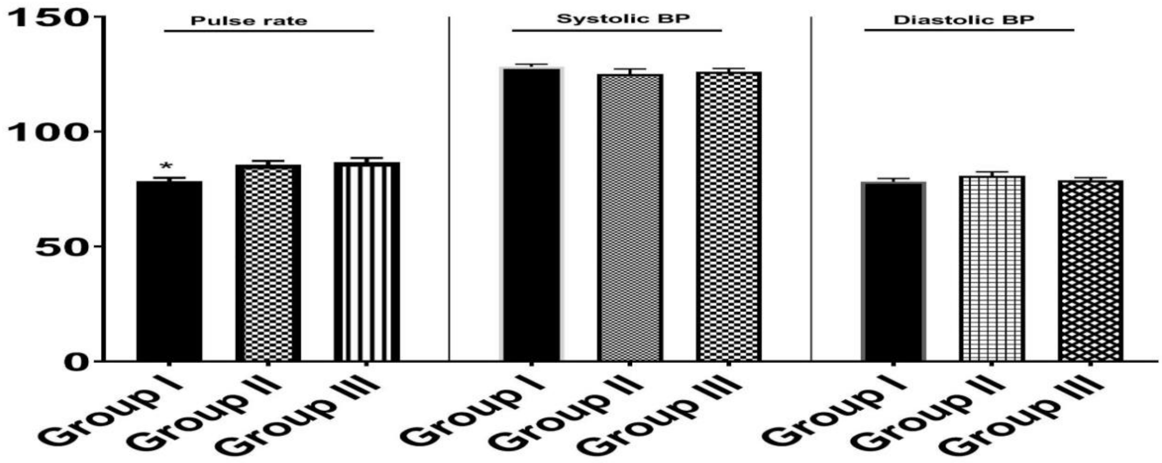

Figure 2. Pulse rate, systolic and diastolic blood pressure in control (Group I), workers (Group II) and peoples live nearby (Group III). Values are expressed as Mean± SEM.; * represent significant difference among groups.

Figure (3) shows a significant increase in serum IL-6 levels among workers (Group II) compared to corresponding level in control (Group I) *(P<0.01)*. Additionally, in comparison to the control (Group I), there was a non-significant increase in serum IL-6 in (Group III).

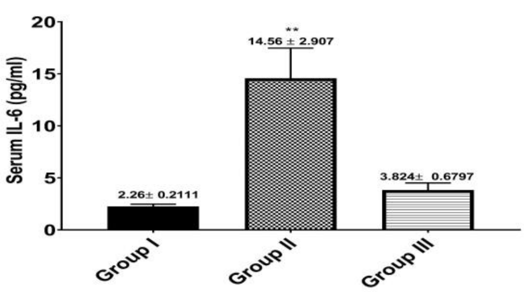

Figure 3. Serum interleukin-6 level of the participants in the study (n=150). Values are expressed as Mean±SEM; *represent significant difference *P<0.01*among groups.

Moreover, levels of RBG were found to be significantly-higher *(P< 0.05)* in workers (Groups II) compared to controls (Group I) (Figure 4).

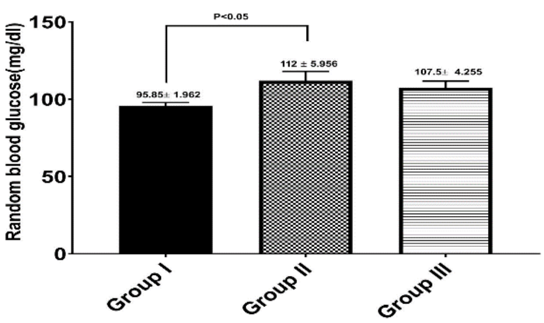

Figure 4. random blood glucose of the participants in the studied groups (n=150). Values are expressed as Mean± SEM. There is significant difference in Group II compared to Group I (P<0.05).

Furthermore, Table 2 illustrated that, each of WBC number, granulocytes numbers and monocyte were significantly-increased in Group II and Group III compared to such numbers in Group I.
In workers (Group II) and those who live near the company (Group III), the red blood cell count, hemoglobin, and HCT were all negatively-related with air pollution [no significant results (P>0.05)]. Moreover, MCV, MCH, and MCHC data provide information on the amount and average concentration of hemoglobin in RBC; where, there was a significant reduction in their values in Groups II and III when compared to such values in Group I/control persons; since, the results of this study show a significant rise in PLTs count in Group II patients in comparison with such count in Group I and Group III. In addition, uncontrolled-activation and distribution were linked to a significant increase in PDW-SD and PDW-CV in Groups II and III compared to that in Group I.

Table 2. Comparison of complete blood count parameters among participants of the studied groups

Groups / **Parameters**	**Group I**	**Group II**	**Group III**	***P-Value***
WBC (10^9/L)	6.47±0.23	7.27±0.21a	7.82±0.27a	***P<0.05***
Lym#(10^9/L)	1.755±0.1264	1.767±0.07504	1.739±0.07951	***P>0.05***
mono#(10^9/L)	0.7663±0.0460	0.8708±0.03537	0.8425±0.04052	***P>0.05***
Gran#(10^9/L)	4.035±0.1877	4.413±0.1893	5.12±0.2201a	***P<0.05***
Lym %	27.52±1.353	26.2±1.299	23.95±0.9021	***P>0.05***
Mono%	11.98±0.5306	12.25±0.3049	10.8±0.3867a	***P<0.05***
Gran %	61.83±1.356	61.88±1.22	65.61±1.075	***P>0.05***
RBCs (10^12/L)	5.325±0.07553	4.81±0.06927	4.842±0.0954a	***P<0.05***
HGB (g/dl)	14.32±0.1616c	13.56±0.1843b	12.31±0.176a	***P<0.05***
HCT %	43.54±0.4532	41.06±0.6995a	43.54±0.4532a	***P<0.05***
MCV	82.44±0.707	79.75±0.7693	69.9±2.032a	***P<0.05***
MCH pg	27.1±0.2526	26.56±0.2802a	26.52±1.108a	***P<0.05***
MCHC g/dl	33.06±0.1247	313.55±0.1705	30.84±0.6313a	***P<0.05***
PLTs (10^9/L)	206.9±8.444	255.1± 3.39a	198.4± 6.5	***P<0.05***
PDW-SD	12.09±0.235	14.87±0.1765b	13.04±0.216a	***P<0.05***
PDW-CV	14.23±0.1417	14.74±0.1422b	14.91±0.1208a	***P<0.05***

Values are expressed as Mean±SEM. Different characters (a, b, c) different letters represent significant difference between groups *P*<0.05. WBC: white blood cell, Lym#: lymphocyte, Mono#: monocyte, Gran#: granulocyte, Lym%: lymphocyte percentage, Mono%: monocyte percentage, Gran%: granulocyte percentage, RBC: red blood cell, HGB: hemoglobin, HCT%: hematocrit percentage, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLTs: platelets, PDW-SD: platelet distribution width-standard deviation, PDW-CV: platelet distribution width-cell volume.

The correlation between serum IL-6 levels and certain hematological indices revealed that each of WBC and PLTs were significantly correlated to IL-6 *($P<0.05$)*; on the other hand, other parameters such as monocytes, lymphocytes, granulocytes, RBC and Hb were non-significantly-correlated (*P*>0.05) as clarified in Table 3.

Table 3. Correlation analysis between serum IL-6 and hematological indices.

	IL-6 vs WBC	IL-6 vs Monocytes	IL-6 vs Lymphocytes	IL-6 vs Granulocytes	IL-6 vs RBC	IL-6 vs Hb	IL-6 vs Platelets
R squared	0.106	0.0009	0.0676	0.068	0.003	0.0004	0.07948
***P* value**	0.020	0.8343	0.0681	0.0660	0.669	0.8883	0.0473
***P* value summary**	*	ns	ns	ns	ns	ns	*

*** Represents significant difference (*P*<0.05); ns represents (non-significant) (P>0.05).**

Discussion

This study was done to explore the impact of oil wells-containing petrol vapor on human inflammatory, hematological markers which are the major criteria for determining hydrocarbon toxicity in humans [15]. In the present study, most participants with direct contact with Oil Wells Refinery were males (80-90) %, due to hard condition and style of the work ladies do not generally-involved as main workers in petrol stations in Iraq. There were significant differences *(P*<0.05*)* among the groups in smoking habit, as Group II presented a greatest percentage of smokers (40%) are seen. Regarding coffee consumption, about 32% of the participants in both Group I/ control and Group II workers drank a lot of coffee. Furthermore, the current study showed that there were no-significant differences in BMI among the individuals of all groups, in contrast to other study, which discovered that exposure to a number of polycyclic aromatic hydrocarbons (PAHs) and volatile organic chemicals (VOCs) was directly-correlated to an elevated risk of obesity (16). Moreover, in the present study, there was a considerable rise in pulse rate in Groups II and III when compared to control, but it was still within normal limits; in contrast, no significant differences in systolic and diastolic blood pressure were found among groups of this study, and the majority of them were within normal ranges. Additionally, previous studies found that the rate of hypertension was substantially- higher in the group with high benzene exposure (100%) than in the group with moderate exposure (49%), this indicating to potential CV involvement of oil exposure or its waste products on people who work or live near to oil wells [17]; moreover, a significant conclusion by other study is that, 1 hour of exposure to essential oil wa associated with higher -blood pressure and -heart rate; furthermore, this finding was also reported by Chuangkj *et al* [18]. A possible mechanism of benzene-induced hypertension could be due to a disturbance of the biological process involving ROSs, which are known to induce lipid peroxidation, decrease antioxidants and increased oxidative stress (OS) which may result in hypertension [17] [19]. The differences between the current study and these previous studies may be due to the differences in the work conditions including concentration of gases and vapor as well as the exposure time of the workers as reported by the study [20]. Actually, petroleum-exposure may stimulate the production of pro-inflammatory IL-6 and decrease the production of anti-inflammatory cytokines like IL-10; furthermore, other investigators concluded that, petroleum products can cause immune system imbalances, because the concentration of cytokines in the circulation can be fluctuated; and thus, it has been used as a marker to assess the deleterious effects of long-term exposure to oil products as major air pollutants [6].

Various inflammatory processes and the production of ROSs are part of the suggested mechanism due to the introduction of hazardous chemicals from petroleum products [11]. The current study shows a significant increase in serum IL-6 level among workers (Group II) compared to such level in control (Group I) *(P*<0.05*)*. The increased of serum IL-6 levels are linked to an increased risk of developing clinical disorders, especially if the level exceeds a predetermined cutoff point; this was in agreement with other study when comparing the workers to the non-exposed group [19]. This is may be due to a variety of pathogenic pathways that can be triggered by long-term exposure to air contaminants [21]. Moreover, it has been found that exposure to a number of PAHs and VOCs was linked to an elevated risk of diabetes [16]. Furthermore, constant exposure to fuel and chemical waste products raised the risk of developing certain endocrine disorders. In our study, the participants' random blood glucose concentrations compared to the control group levels were significantly-higher *($P<0.05$)* in workers of (Group II) compared to healthy individuals in the control (Group I), this difference could be attributed to the differences in the work conditions and lifestyle including consumption of more coffee with sugar and uncontrolled diet. However, the current finding revealed that random blood glucose in all groups was within normal limits. On the other hand, other study found that urine poly-aromatic hydrocarbon (PAHs) were linked to a higher incidence of elevated glucose levels than other group without contact with PAHs [22]. Generally, the endocrine involvement of chronic exposure to fuel waste products or air pollution may be the end results of subsequent organs dysfunctions [23].

The results of hematological parameters in this study, showed significant differences in each WBC, [granulocytes and monocytes (%)] among the studied Groups; where, the numbers of these parameters were significantly-increased in Group II and Group III compared to that in Group I. Furthermore, the RBCs, Hb, and HCT were negatively-related to air pollution in Group II and Group III. Moreover, there was a significant reduction in MCV, MCH, and MCHC values in Groups II and III when compared to such values in Group I; as well as, there was a significant rise in PLTs count in Group II persons in comparison with that count in Group III and Group I. This variation might be attributed to that continuous exposure to petroleum products that may cause a reduction in the function of BM and phagocytic cell migration failure [24] [25]; and there were signs of OS and inflammation, as well as increased ROSs production, which can lead to cytotoxicity and increase PLTs count [26]. Other previous study found that, the oil worker attendants had much lower Hb levels and red blood cell (RBC counts) than that in the control (Group I); but, their mean white blood cells (WBCs) and PLTs counts were greater; additionally, the MCV, MCH, and MCHC were similar in both groups [25]. The current study showed that anemia and leukopenia were the most common hematological-abnormalities as a result of benzene exposure at oil refinery. Previous studies found that workers who were exposed to vapors of oil for 2 years or longer had significantly-lower HGB, RBC, MCV, and MCHC levels [6] [25]; and the adverse effect of oil exposure on several hematological indices and IL-6 as inflammatory marker may be related to certain inflammatory processes.

Conclusion

Workers who have worked at Oil Refinery for at least two years might develop several hematological disorders including anemia. Also, these workers are more likely to be at risk of certain inflammatory processes and changes in which IL-6 is involved. Therefore, workers at these certain workplaces should be aware of the consequences of working in these places and should do clinical routine checkup to avoid and treat any health problem that may occur.

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